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Fig. 1: Concentration of OPG in human breast milk at different times during lactation

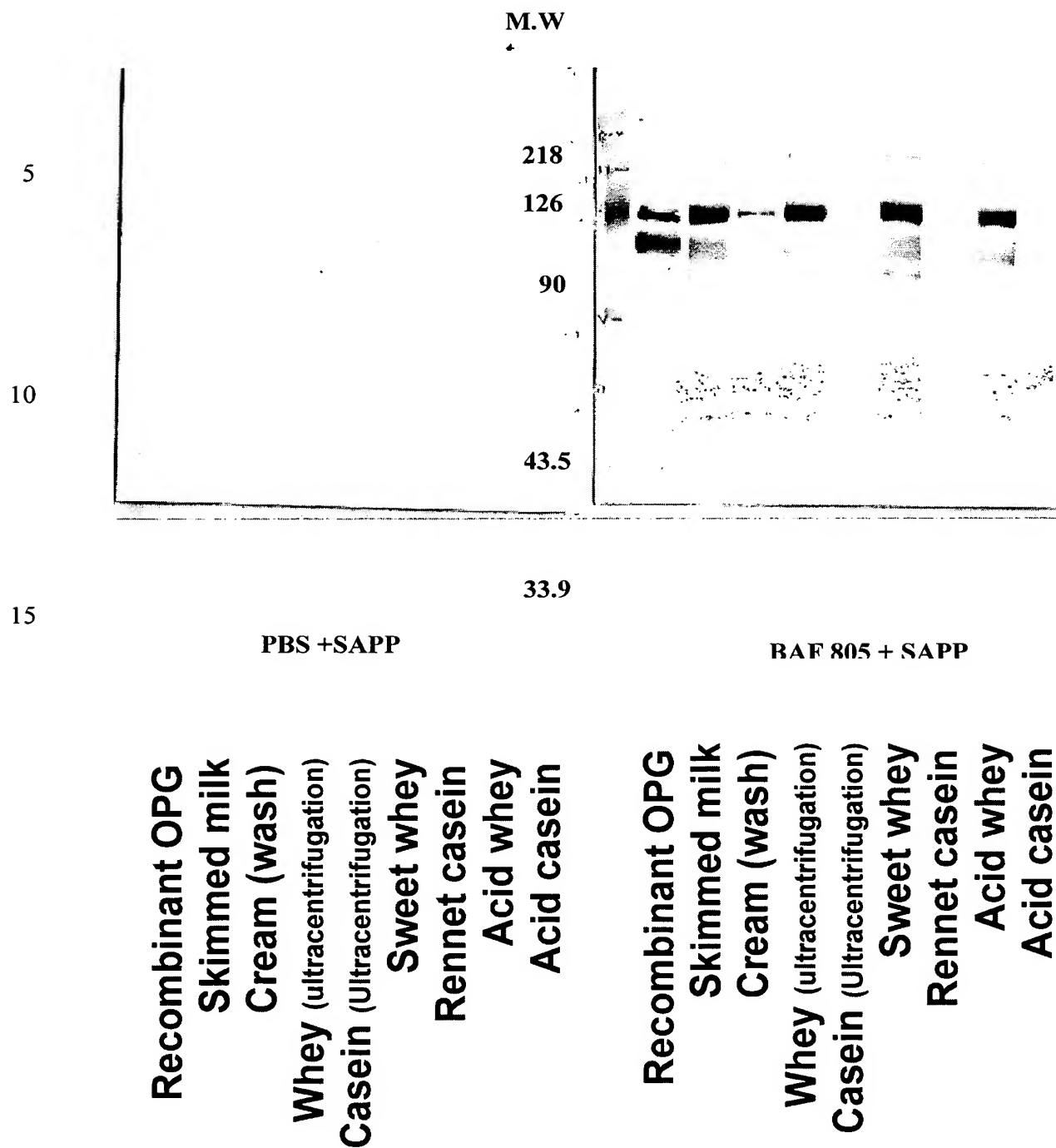


Fig. 2: Western blot analysis of human milk fractions under reducing conditions using 10% SDS-gel. Bands for OPG were revealed using the biotinylated anti-OPG polyclonal antibody, BAF805 from R&D Systems and streptavidin-alkaline phosphatase (SAPP).

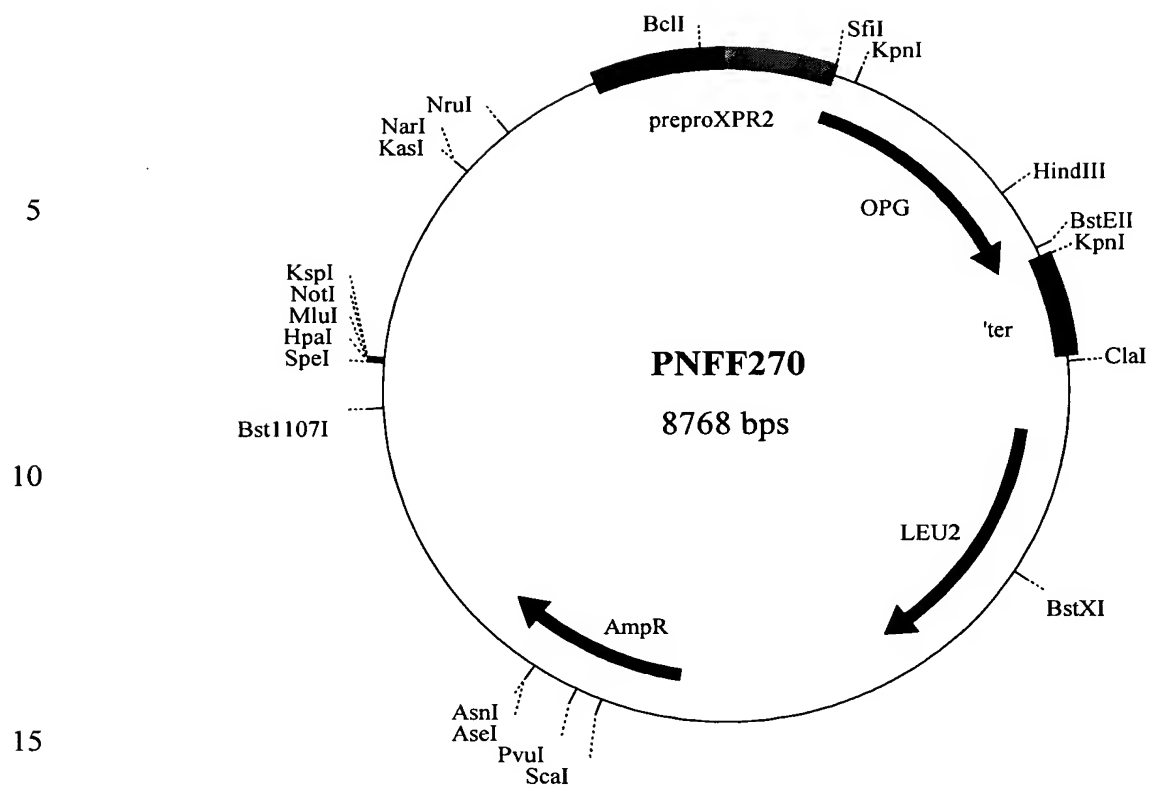


Figure 3. The restriction map of the plasmid which was integrated into the genomic DNA of

20 *Yarrowia* transformants.

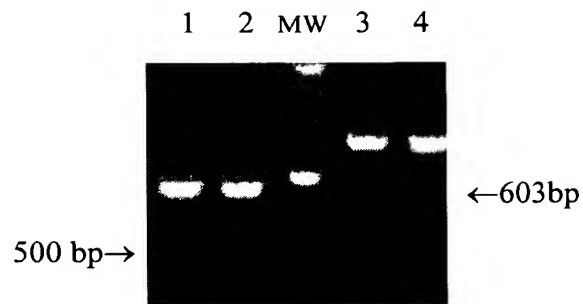


Figure 4. RT-PCR analysis of human breast milk cells and human mammary gland epithelial cells, MCF-7.

Lanes 1 and 2 : $\beta$ -actin (expected size band: 460 bp)

Lanes 3 and 4 : OPG (expected size band: 603 bp)

1. Human breast milk cells

2. MCF-7

3. Human breast milk cells

4. MCF-7

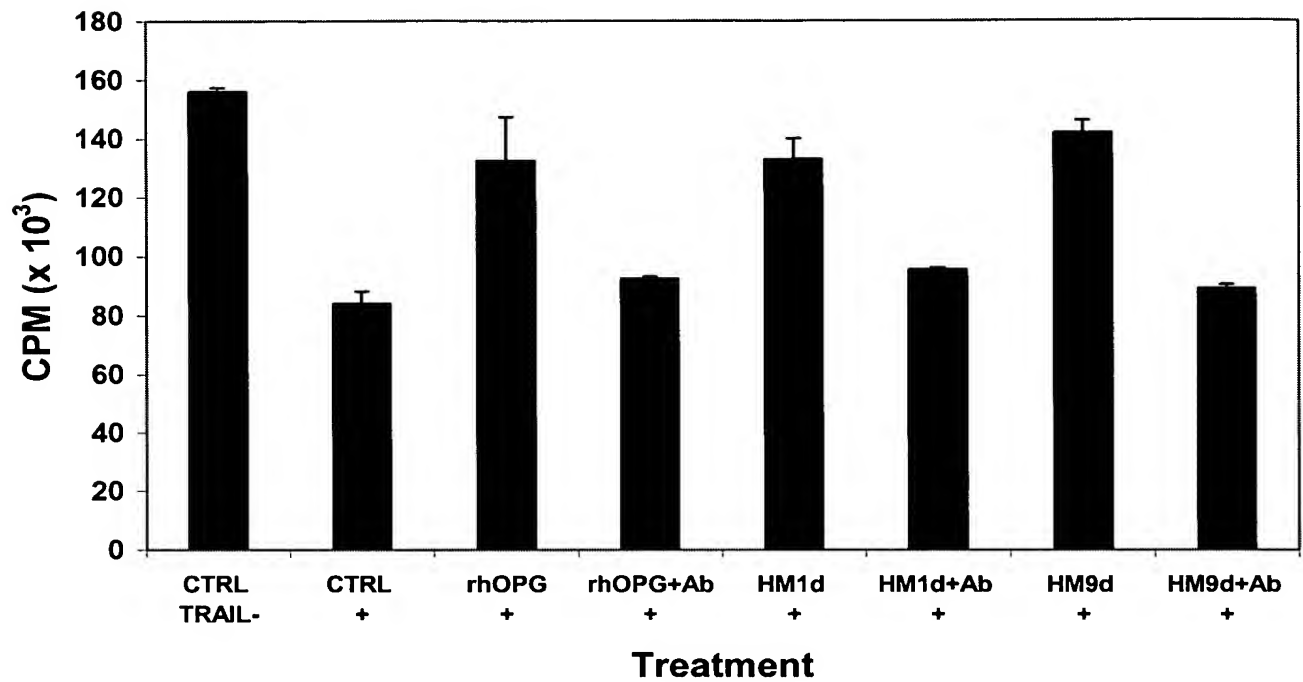


Figure 5: OPG inhibits TRAIL-induced apoptosis of Jurkat cells. The figure depicts a single representative experiment of Jurkat cells treated with 20ng/ml of TRAIL and recombinant human OPG (rhOPG) or human milk (HM) at a final dilution of 1/80. The HM in the above experiment was from a single mother at either 1 day or 9 days postpartum. Antibody (Ab) against OPG was used at a concentration of 20µg/ml. Cell proliferation was measured by <sup>3</sup>H-thymidine incorporation. In control wells (CTRL), cells were exposed to culture medium with or without TRAIL.

MKLATAFTILTAVLAAPLAAPAPAPDAAPAAPVPEGPAAAAYSSILSVVAKQSKKFHHKR  
DLDEKDQFIVVFDSSATVDQIASEIQKLDLVDSSNGITSALDLPVYTDGSGFLGFVG  
KFNSTIVDKLKESSVLTVEPDTIVSLPEIPASSAAKRETFPPKYLHYDEETSHQLLCDKC  
PPGTYLKQHCTAKWKTVCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRV  
CECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHT  
NCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVTLCEEAFRFAVPTKFTPNWLSVLV  
DNLPGTKVNAESVERIKRQHSSQEQTFQLLKLWKHQKAQDIVKKIIQDIDLCENSVQRH  
IGHANLTFEQLRSLMESLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKG  
LMHALKHSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL

Figure 6. The protein encoded by the OPG plasmid inserted in *Y. Lipolytica*.

The mature OPG is indicated in bold print.

	1	TCCGGCCTCTTCGGCCgccaagcgaGAAACGTTTCCTCCAAAGTACCTTCATTATGACGA	60
	1	<i>xpr2</i> E T F P P K Y L H Y D E	12
5	61	AGAAACCTCTCATCAGCTGTTGTGTGACAAATGTCCTCCTGGTACCTACCTAAAAACAACA	120
	13	E T S H Q L L C D K C P P G T Y L K Q H	32
	121	CTGTACAGCAAAGTGAAGACCGTGTGCGCCCCTTGCCCTGACCACTACTACACAGACAG	180
	33	C T A K W K T V C A P C P D H Y Y T D S	52
10	181	CTGGCACACCAGTGACGAGTGTCTATACTGCAGCCCCGTGTGCAAGGAGCTGCAGTACGT	240
	53	W H T S D E C L Y C S P V C K E L Q Y V	72
	241	CAAGCAGGAGTGCAATCGCACCCACAACCGCGTGTGCGAATGCAAGGAAGGGCGCTACCT	300
15	73	K Q E C N R T H N R V C E C K E G R Y L	92
	301	TGAGATAGAGTTCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTGGAGTGGTGCAAGC	360
	93	E I E F C L K H R S C P P G F G V V Q A	112
	361	TGGAACCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTTCTTCTCAAATGA	420
20	113	G T P E R N T V C K R C P D G F F S N E	132
	421	GACGTCATCTAAAGCACCCCTGTAGAAAAACACACAAATTGCAGTGTCTTTGGTCTCCTGCT	480
	133	T S S K A P C R K H T N C S V F G L L L	152
25	481	AACTCAGAAAGGAAATGCAACACACGACAAATATGTTCCGAAACAGTGAATCAACTCA	540
	153	T Q K G N A T H D N I C S G N S E S T Q	172
	541	AAAATGTGGAATAGATGTTACCCTGTGTGAGGAGGCATTCTTCAGGTTTGCTGTTCCTAC	600
30	173	K C G I D V T L C E E A F F R F A V P T	192
	601	AAAGTTTACGCCTAACTGGCTTAGTGTCTTGGTAGACAATTTGCCTGGCACCAAAGTAAA	660
	193	K F T P N W L S V L V D N L P G T K V N	212
	661	CGCAGAGAGTGTAGAGAGGATAAAACGGCAACACAGCTCACAAGAACAGACTTTCCAGCT	720
35	213	A E S V E R I K R Q H S S Q E Q T F Q L	232
		C	
	721	GCTGAAGTTATGGAAACATCAAAACAAAGACCAAGATATAGTCAAGAAGATCATCCAAGA	780
	233	L K L W K H Q N K D Q D I V K K I I Q D	252
		A	
40	781	TATTGACCTCTGTGAAAAACAGCGTGCAGCGGCACATTGGACATGCTAACCTCACCTTCGA	840
	253	I D L C E N S V Q R H I G H A N L T F E	272
	841	GCAGCTTCGTAGCTTGATGGAAAGCTTACCGGGAAAGAAAGTGGGAGCAGAAGACATTGA	900
45	273	Q L R S L M E S L P G K K V G A E D I E	292
	901	AAAAACAATAAAGGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCTCAGTTTGTGGCG	960
	293	K T I K A C K P S D Q I L K L L S L W R	312
	961	AATAAAAAATGGCGACCAAGACACCTTGAAGGGCCTAATGCACGCACTAAAGCACTCAAA	1020
50	313	I K N G D Q D T L K G L M H A L K H S K	332

Figure 7. Sequence of milk OPG.

	1021	GACGTACCACTTTCCCAAACTGTCACTCAGAGTCTAAAGAAGACCATCAGGTTCTTCA	1080
5	333	T Y H F P K T V T Q S L K K T I R F L H	352
	1081	CAGCTTCACAATGTACAAATTGTATCAGAAGTTATTTTAGAAATGATAGGTAACCAGGT	1140
	353	S F T M Y K L Y Q K L F L E M I G N Q V	372
10	1141	CCAATCAGTAAAAATAAGCTGCTTATAACTAGTATCACTAGT	1182
	373	Q S V K I S C L	380

Figure 7. Sequence of milk OPG. (Continued)